Clinical Biosensors: An interfacing Challenge and a Materials Question

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So how much more chemistry do we really need?

IRC in Biomedical Materials

Founded 1992

EPSRC Core Grant

Focus on Biomaterials

Cross-disciplinary culture

Materials, Engineering, Dentistry

17 senior staff

Why biosensors?

Direct transduction

(Bio)selectivity

Simple, monolithic structures

Miniaturised

Electrical/optoelectronic readout

Continuous monitoring

Deskilled use

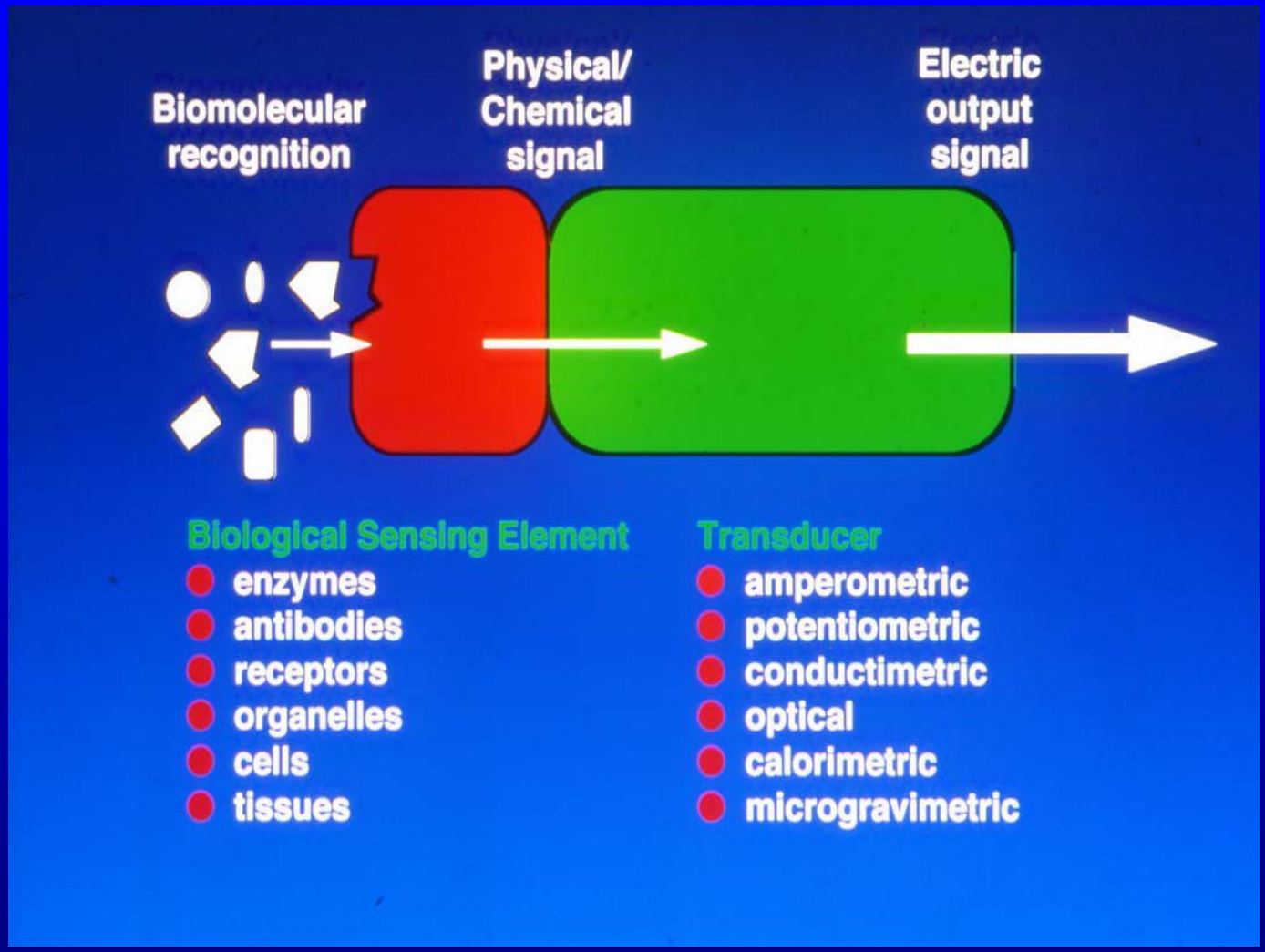
in vivo / ex vivo / in vitro

POCT

Tissue + blood monitoring

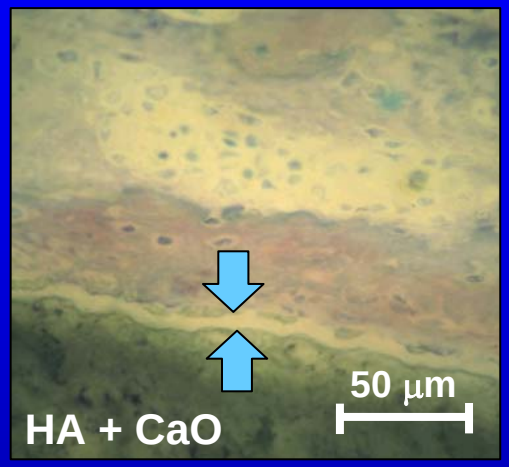
User advantages

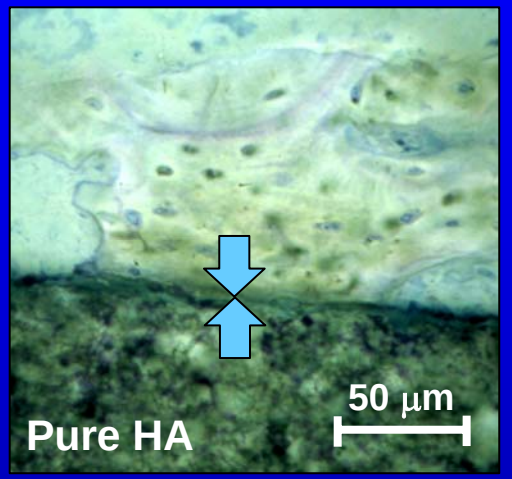
- Consumer commodity
- Medical ‘bypass’
- Cheap
- Reliable
- No sample preparation
- Disposable
- Clean technology


Biomolecular recognition
Physical/ Chemical signal
Electric output signal
Biological Sensing Element
enzymes
antibodies
receptors
organelles
cells
tissues
Transducer
amperometric
potentiometric
conductimetric
optical
calorimetric
micrograviметric

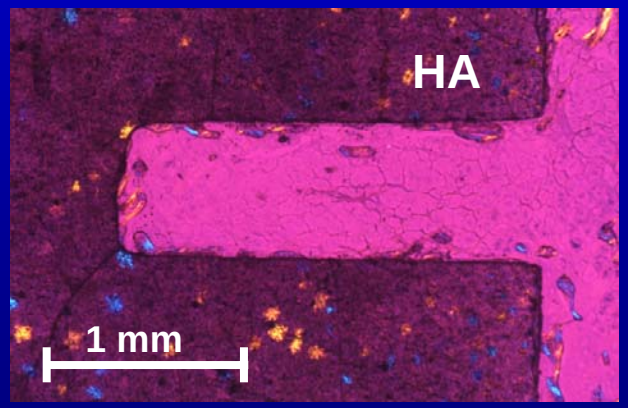
The right chemistry ?

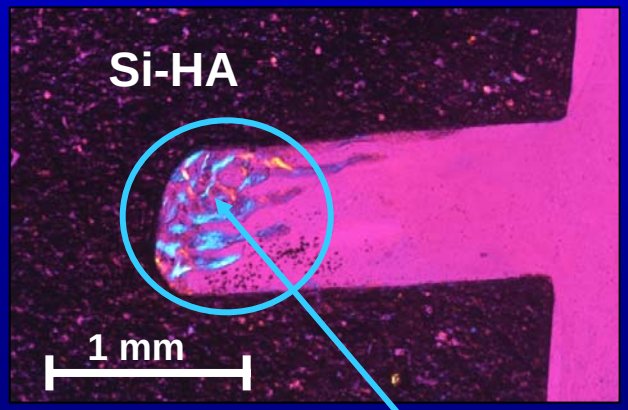
HA Purity





Ionic Substitution





Bone ingrowth (3 week timepoint)

Surface modification of biosensors

Modification of materials interfacial properties in contact with biofluids in order to:

- **Create a selective barrier**
- **Allow transport of targeted analyte**
- **Reduce fouling and maintain performance**
- **Improve long term biocompatibility (inflammation/coagulation)**

Materials used

Organic polymer membranes

- Cellulose acetate
- Poly(Vinyl Chloride)
- Nafion®

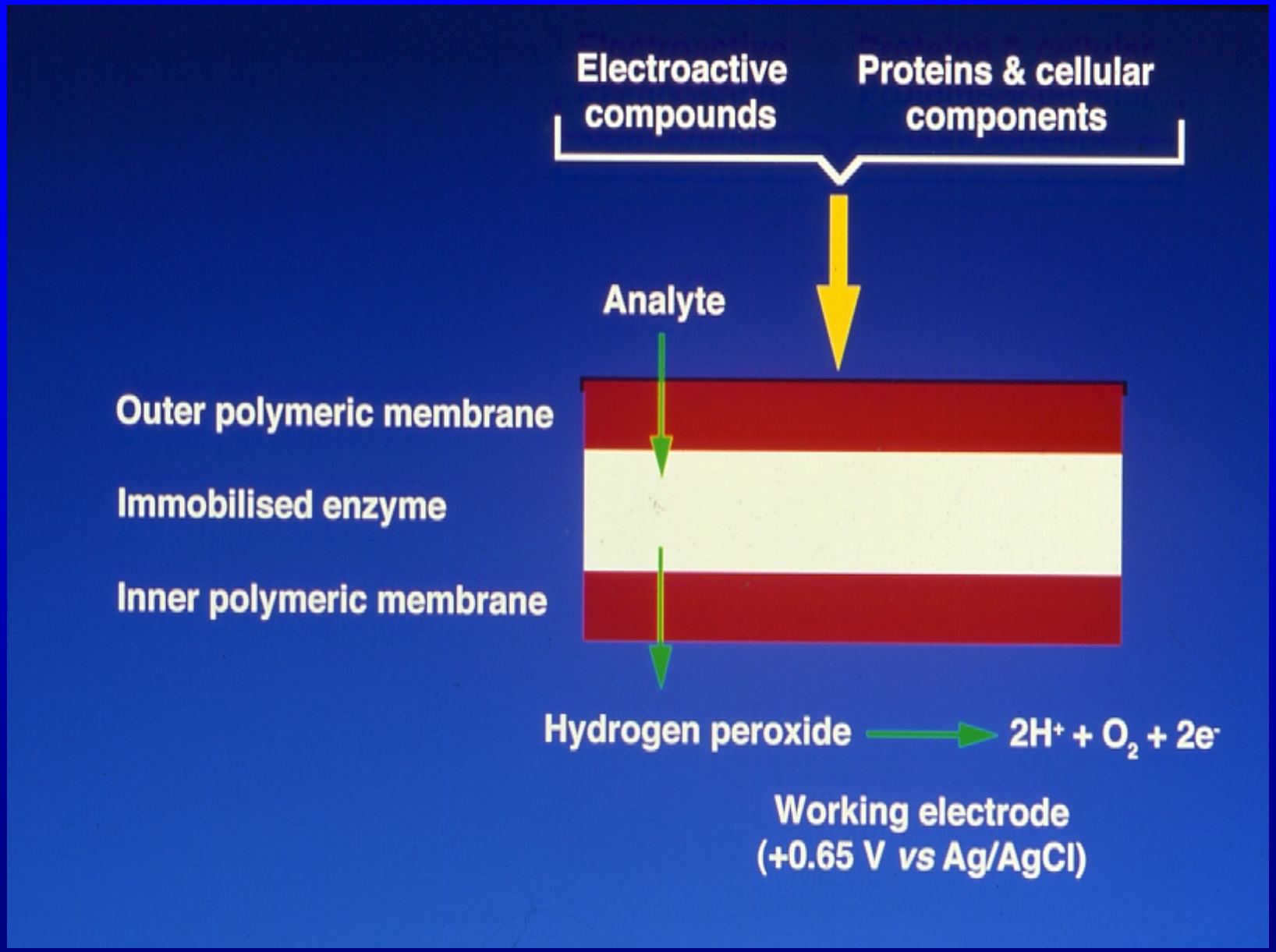
Self-assembled monolayers

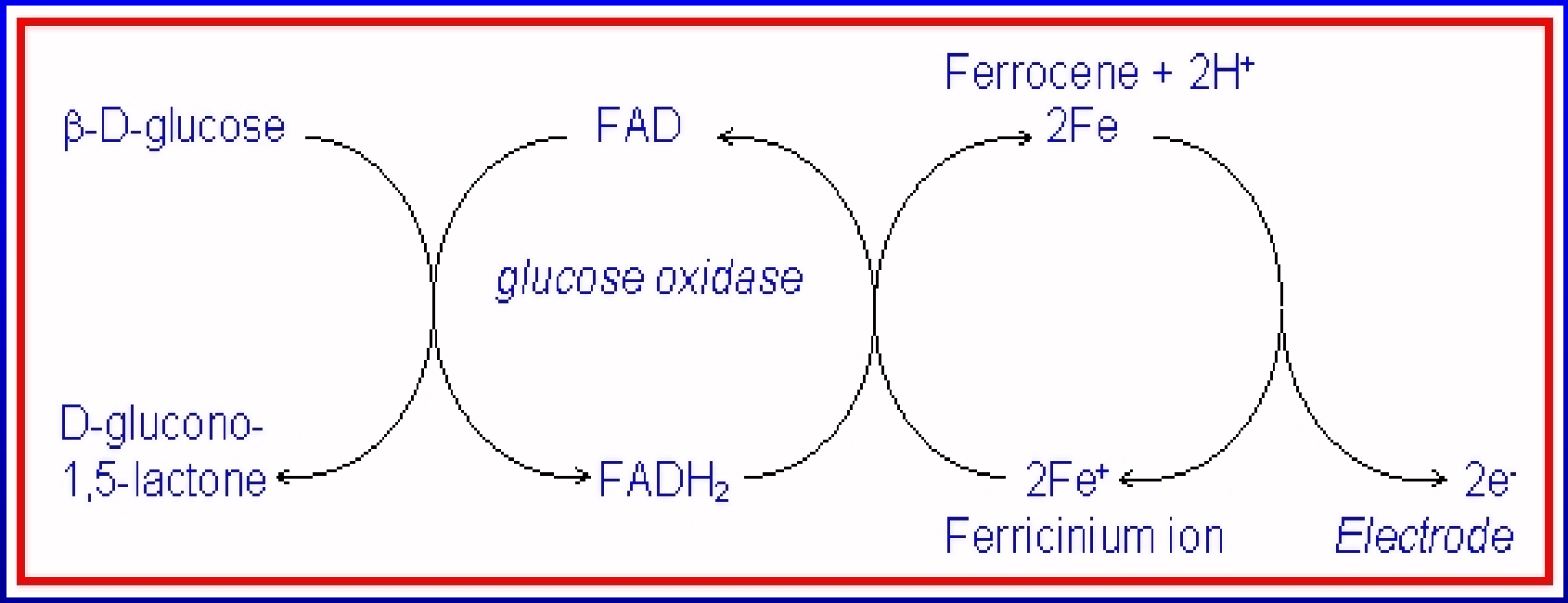
- Alkyl thiols
- Organosilanes

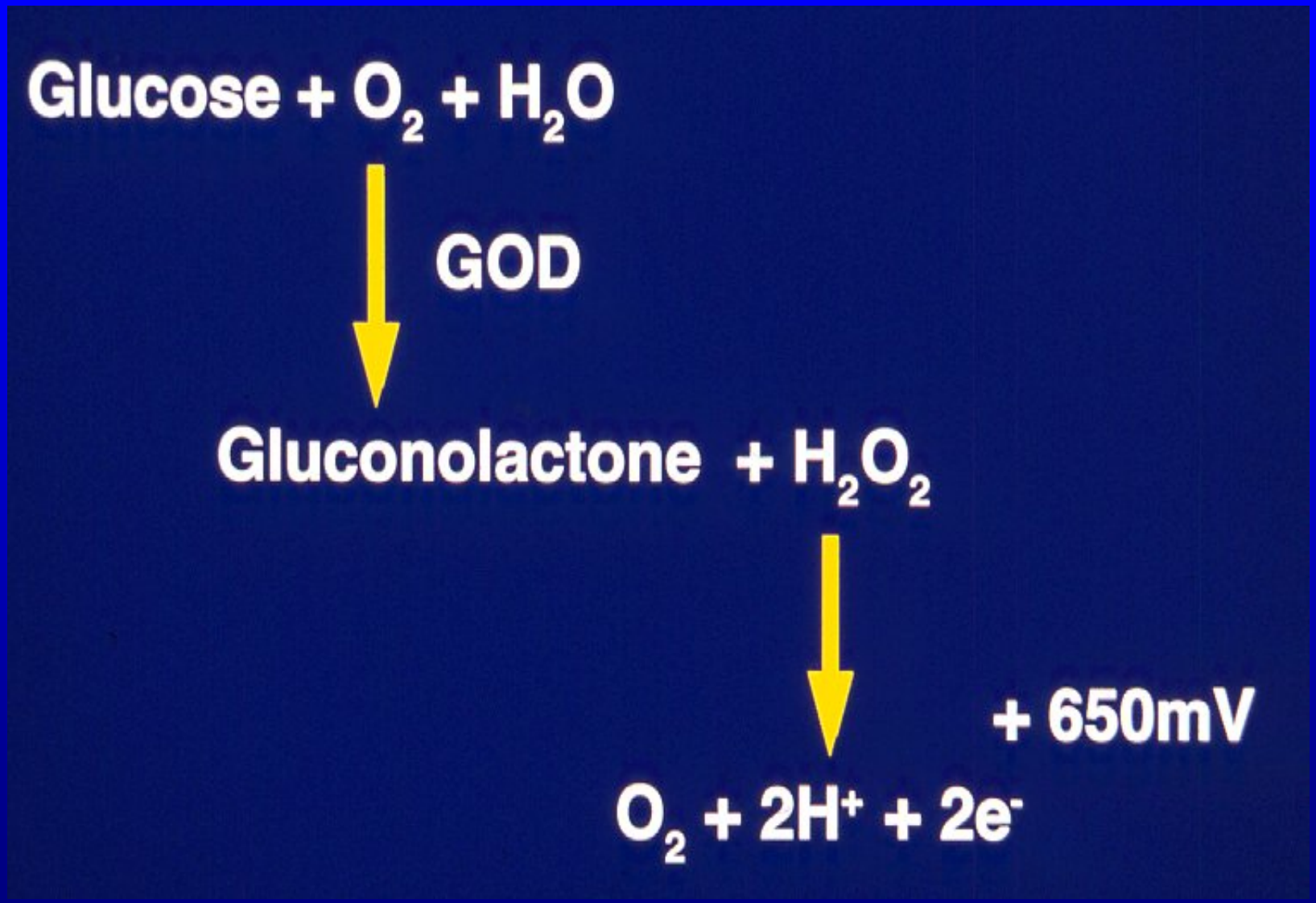
Polymer membranes for biosensor interfacing

Classification of polymer membranes:

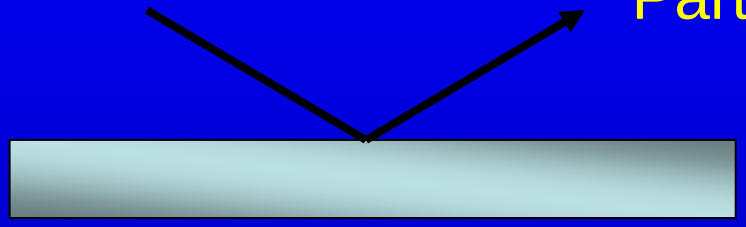
- **Polymeric constituents**
- **Structural anisotropy**
- **Pore size**
  (Provides aperture control on biosensors)


Electroactive compounds
Proteins & cellular components
Analyte
Outer polymeric membrane
Immobilised enzyme
Inner polymeric membrane
Hydrogen peroxide
2H+ + O2 + 2e-
Working electrode
(+0.65 V vs Ag/AgCl)


β-D-glucose
FAD
Ferrocene + 2H+
2Fe
glucose oxidase
D-glucono-
1,5-lactone
FADH2
2Fe+
Ferricinium ion
2e-
Electrode

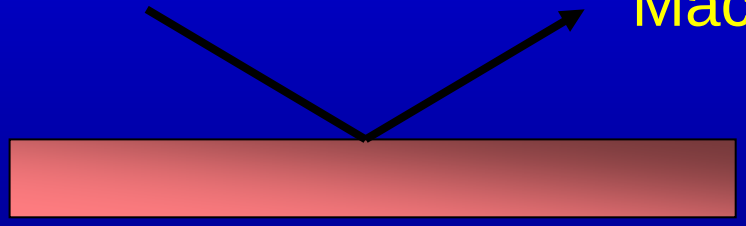

Glucose + O_2 + H_2O
GOD
Gluconolactone + H_2O_2
+ 650mV
O_2 + $2H^+$ + $2e^-$

Membrane technology



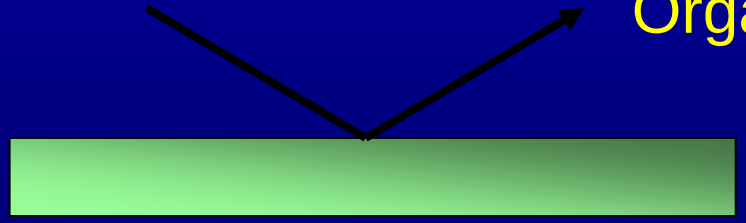
Particles, cells (≥ 50 nm)

MICROFILTRATION



Macromolecules, colloids (≥ 5 nm)

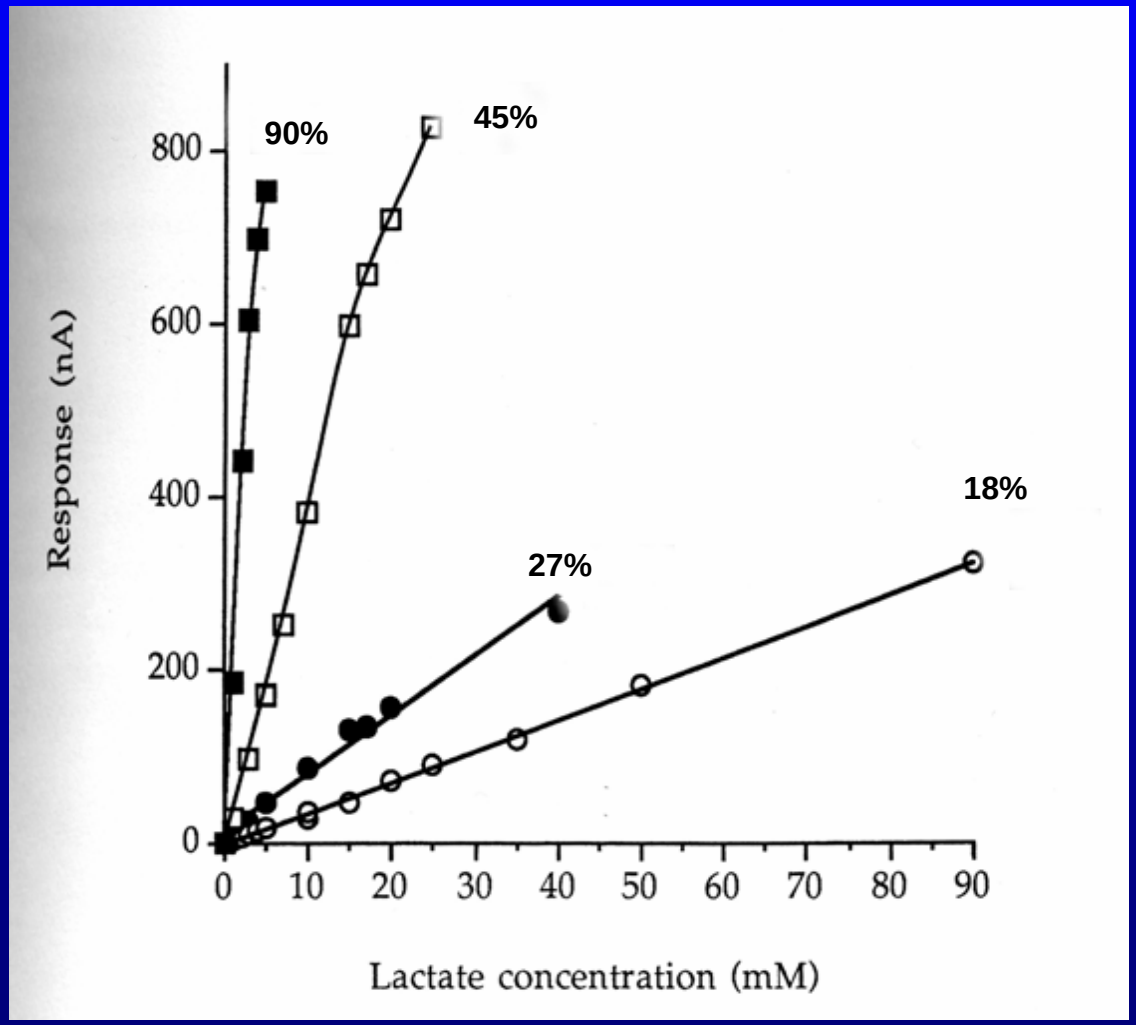
ULTRAFILTRATION

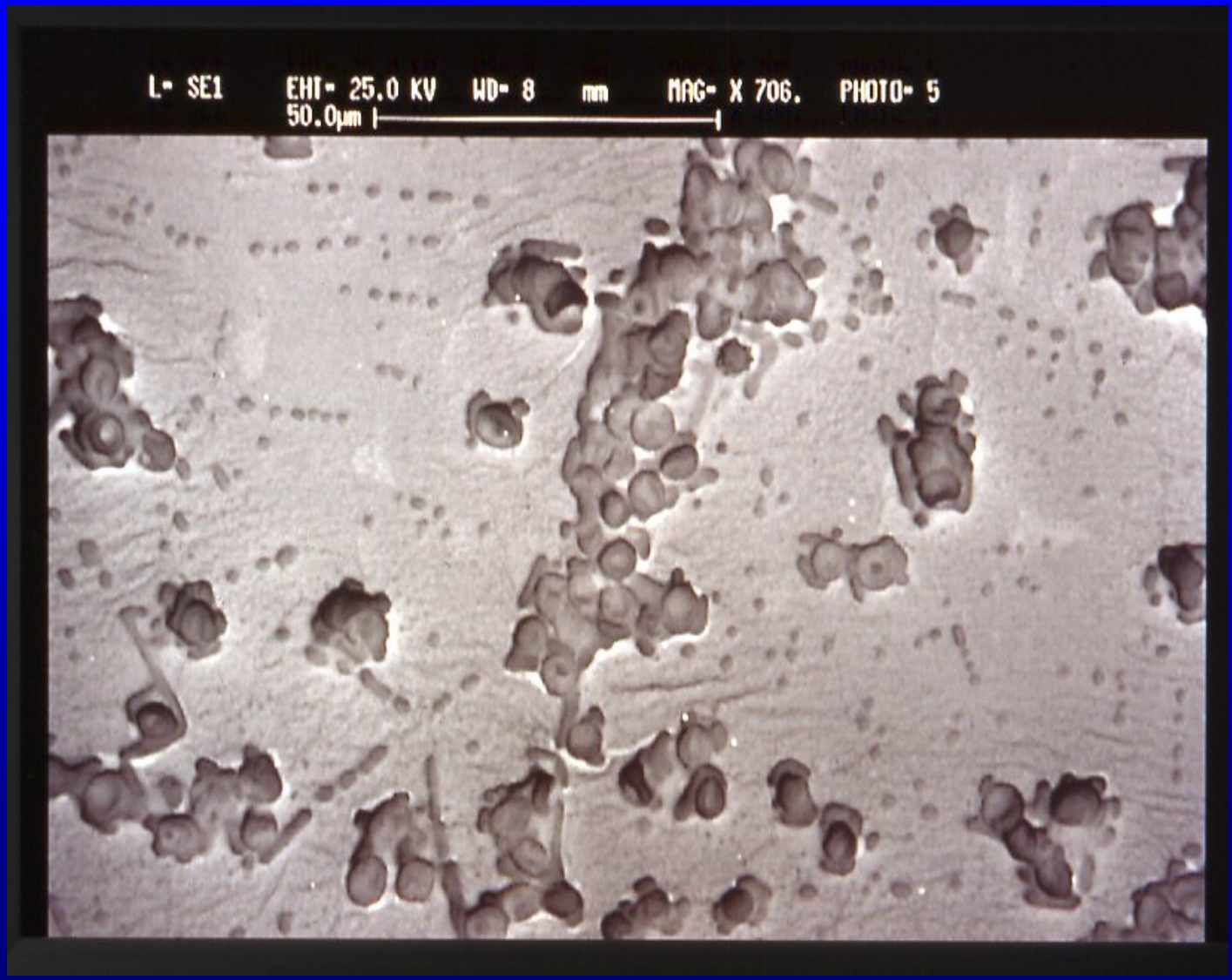


Organics of high M.W. (≥ 0.5 nm)

REVERSE OSMOSIS

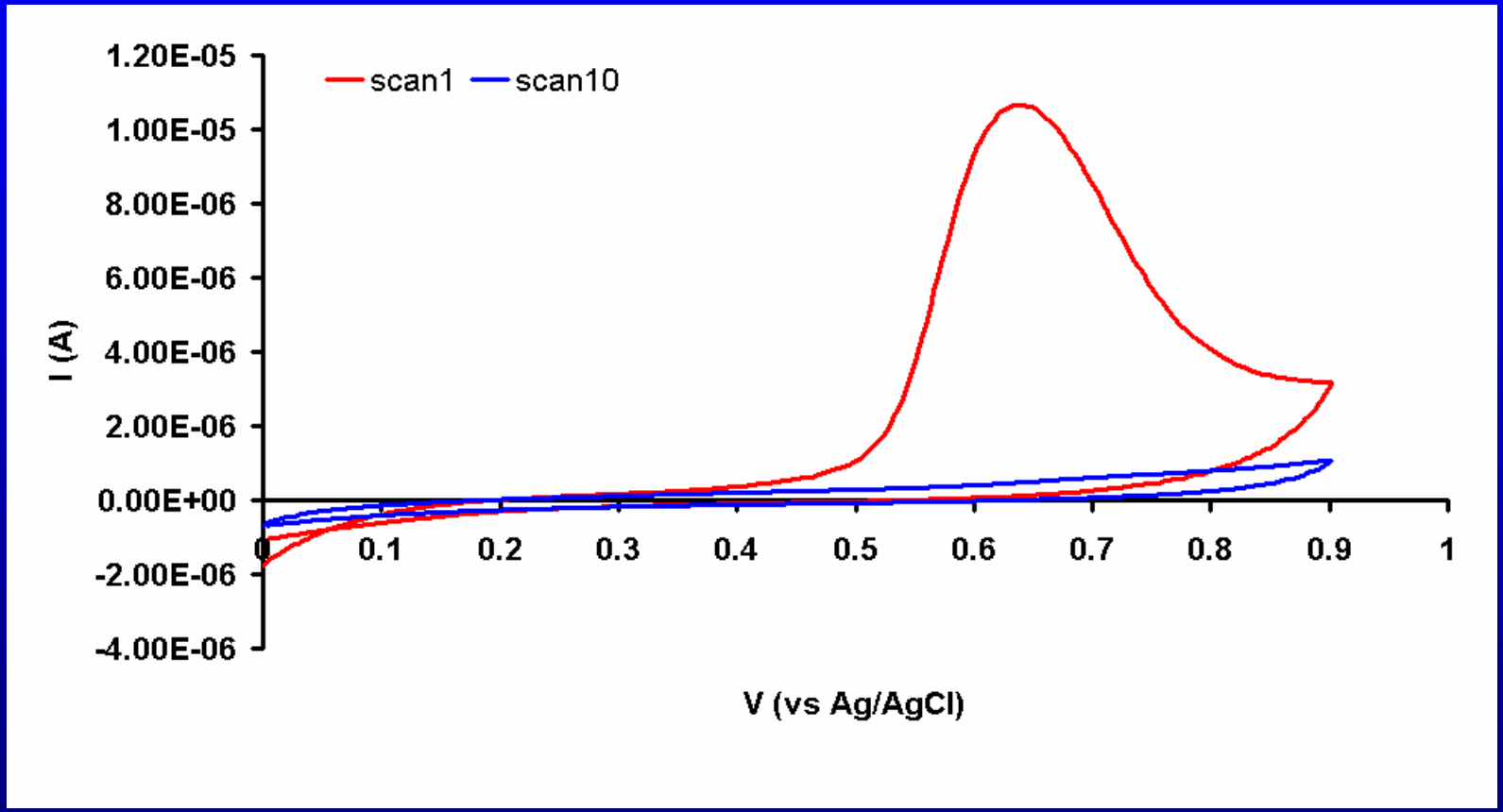
Calibration of lactate enzyme electrode with outer, cast PVC membranes incorporating different amounts of Triton X-100




L= SE1 EHT= 25.0 KV WD= 8 mm MAG= X 706. PHOTO= 5
50.0µm

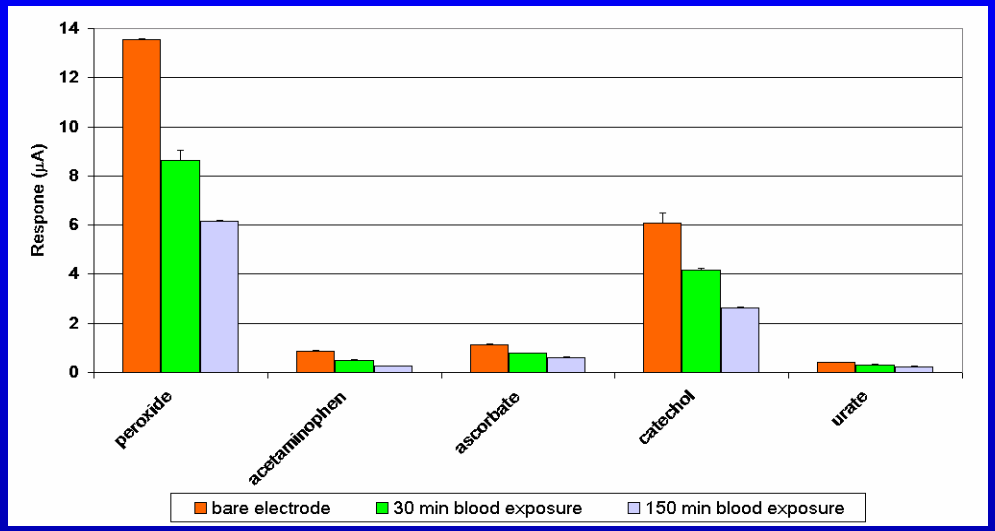
Formation of poly (phenol) on Pt electrode

(5mM phenol, pH7.4, 50mV/s)

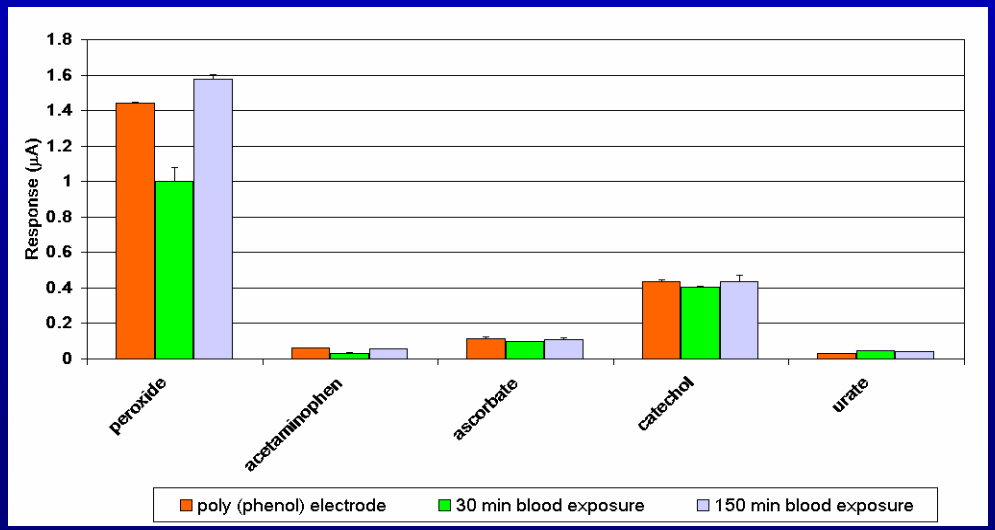


Effect of whole blood on (a) bare (b) poly (phenol)

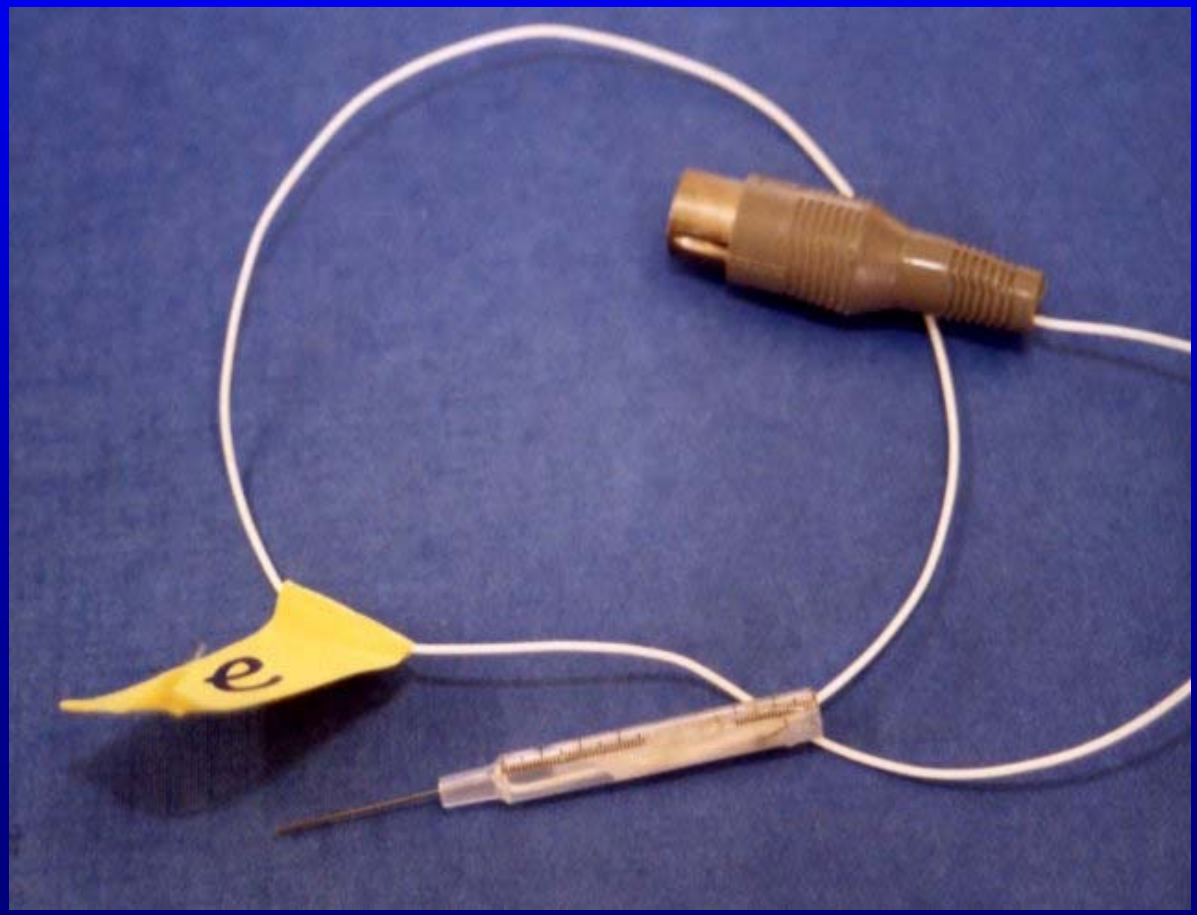
(a)



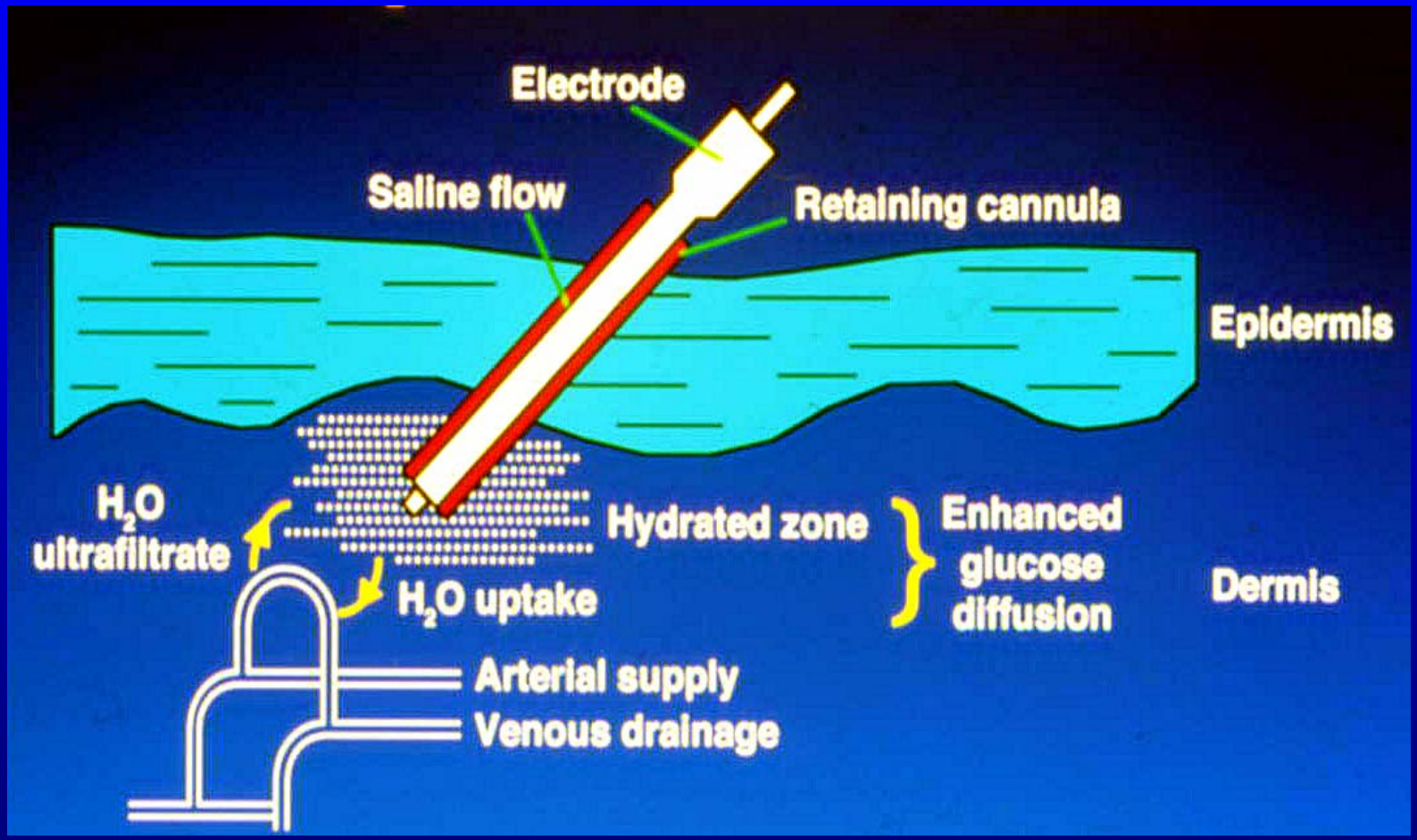
(b)



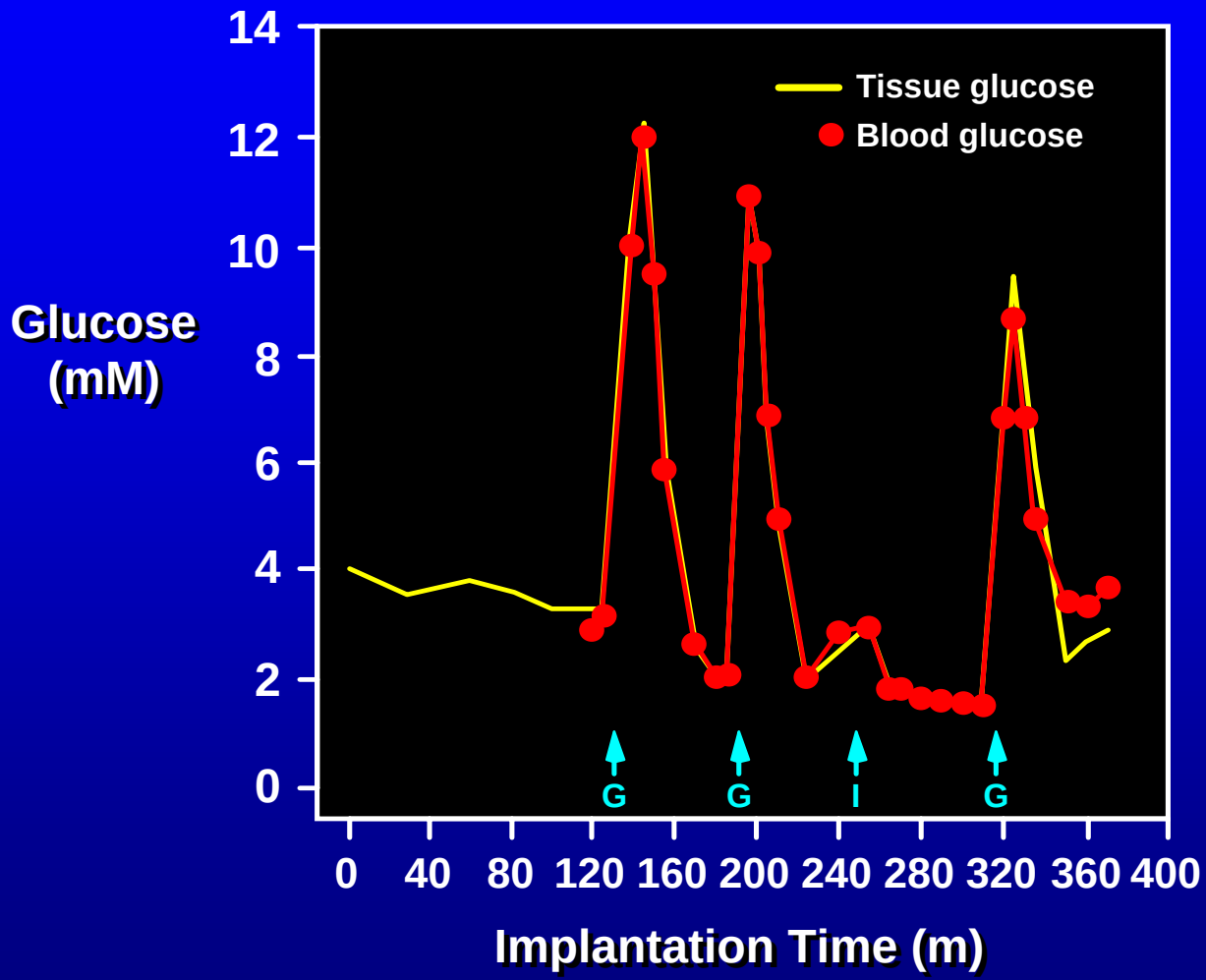
Needle electrode



Open microflow



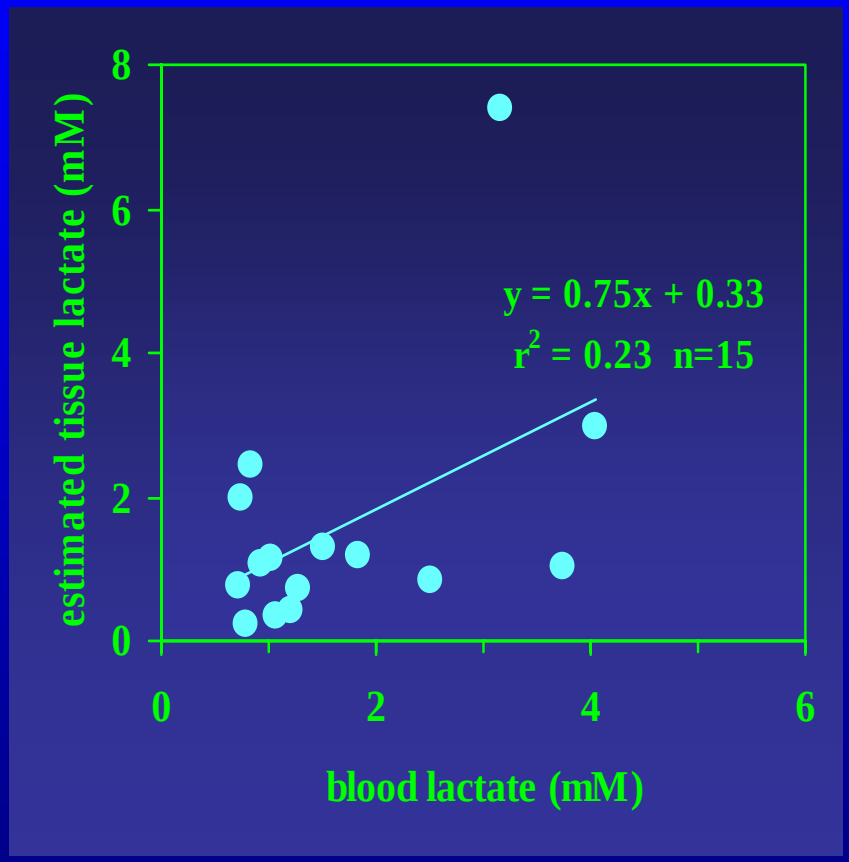
Continuous in-vivo glucose monitoring using isotonic phosphate buffer (pH 7.4) microflow



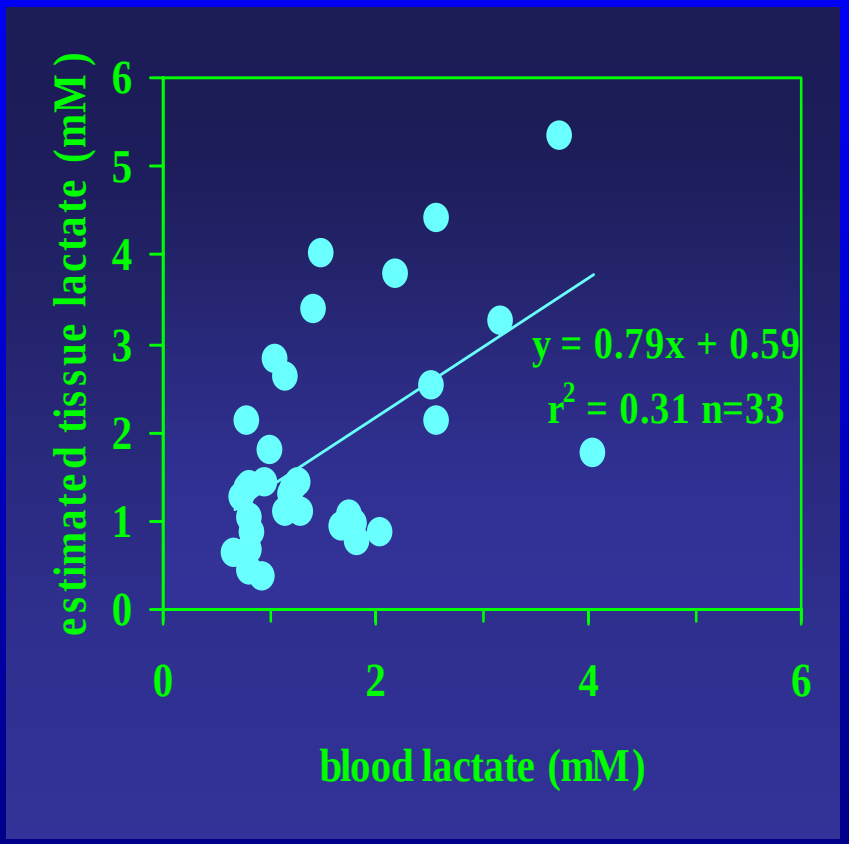
G = Glucose infusion

I = Insulin infusion

Blood-tissue lactate correlation

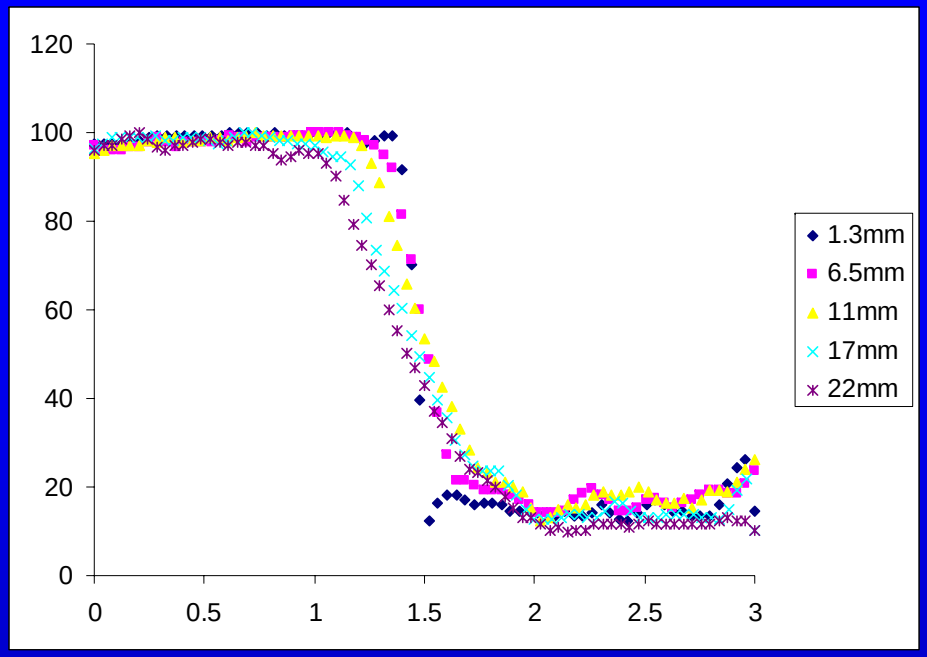


with microflow
5 electrodes

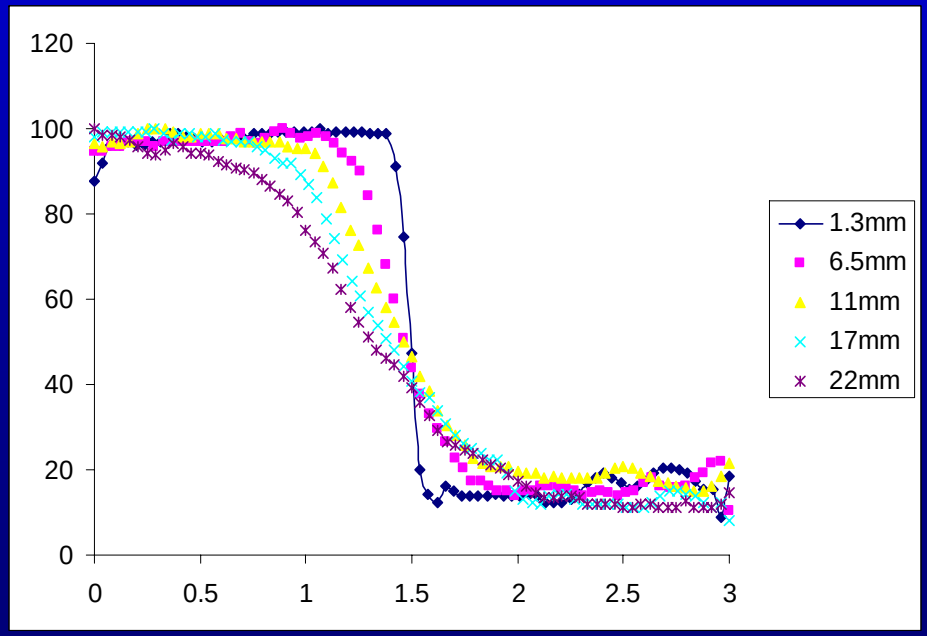


without microflow
11 electrodes

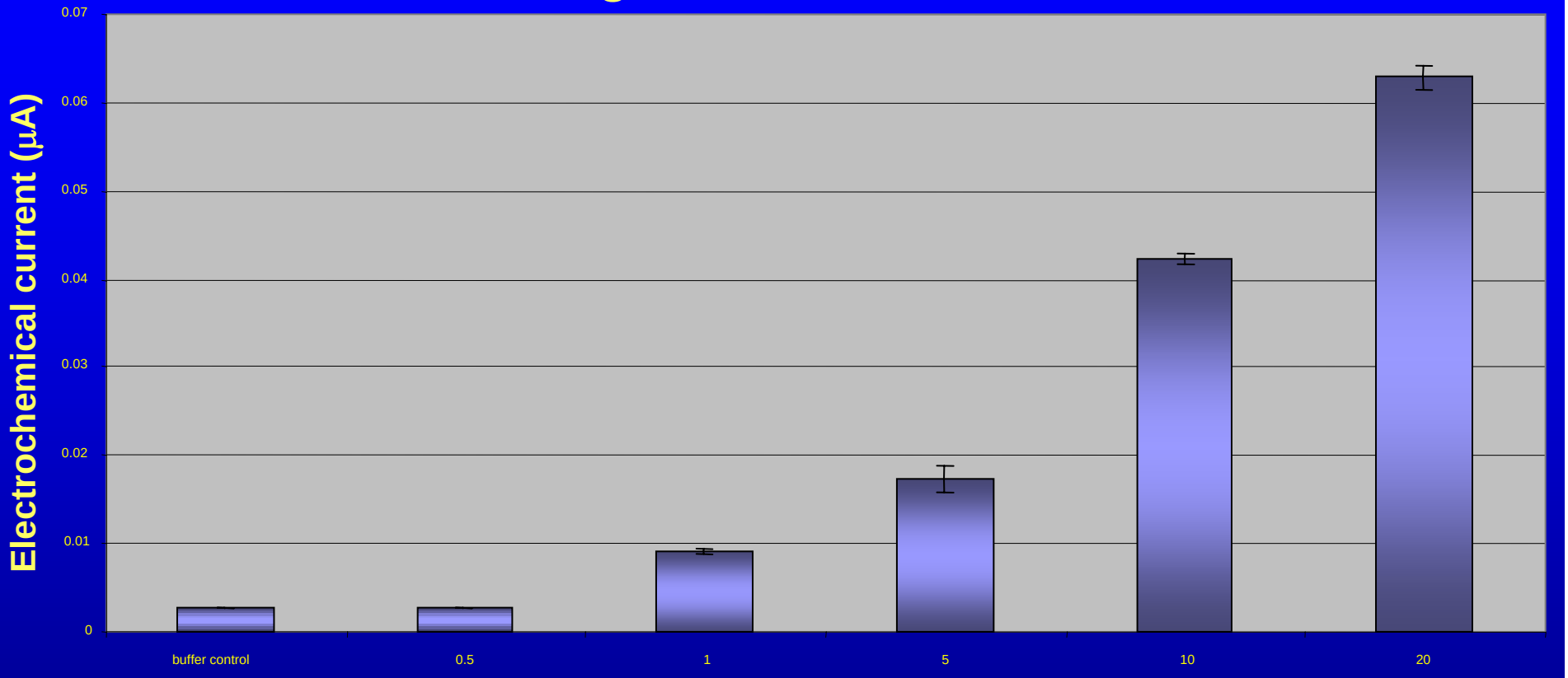
Flow rate = 500 µl/min

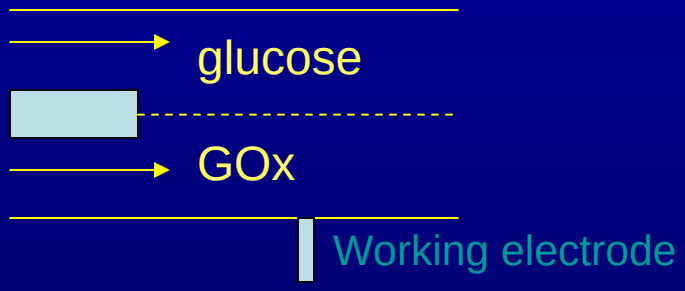


Flow rate = 300 µl/min

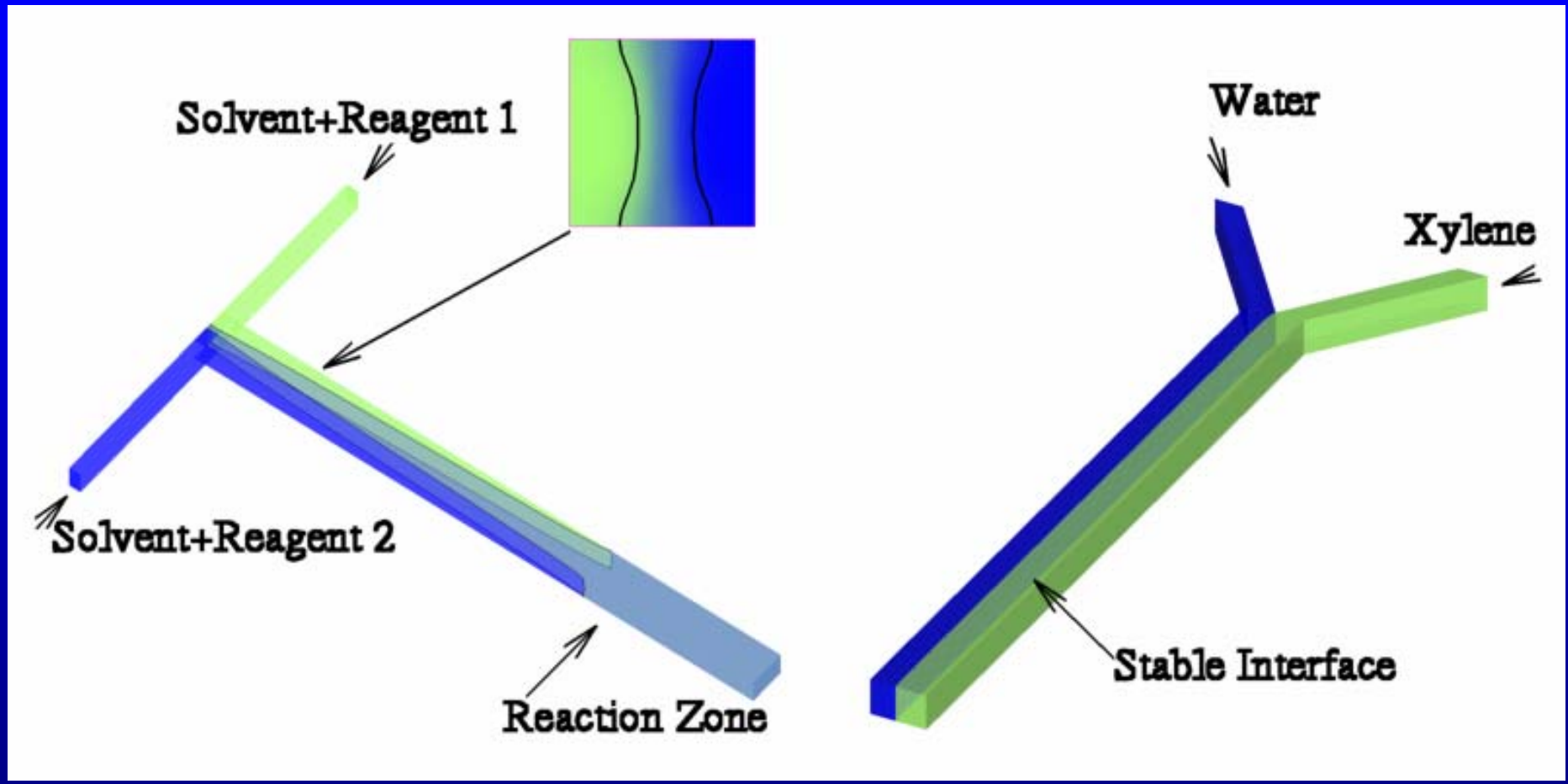


Detection of various glucose concentrations in the indirect stream with 1mg/ml GOx in the direct stream

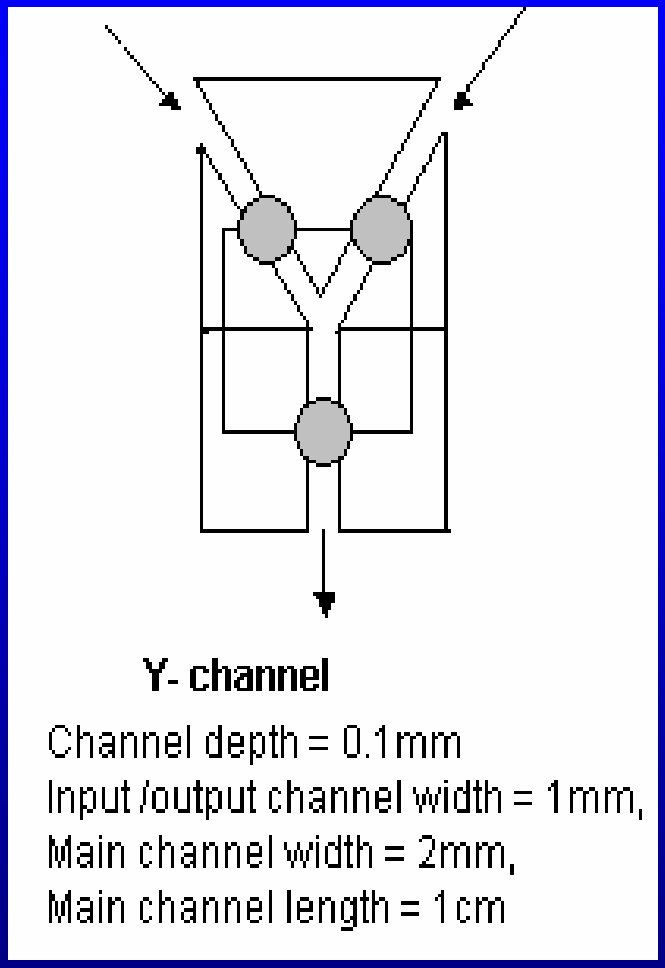




Organic – aqueous interface for polymer formation

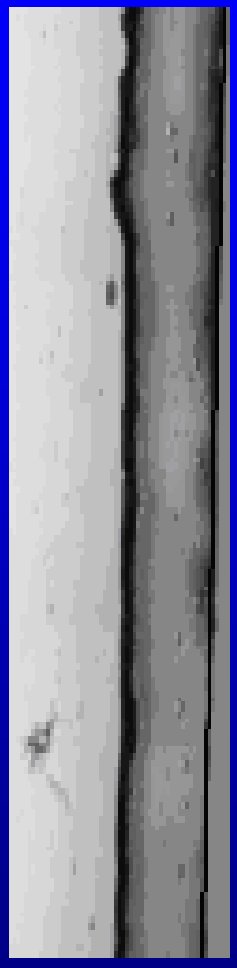


Single Y channel



Y- channel

Channel depth = 0.1mm
Input /output channel width = 1mm,
Main channel width = 2mm,
Main channel length = 1cm



Smoother entry angle

Absence of over- and backpressure

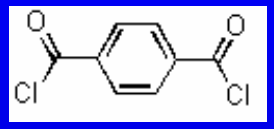
Thin, continuous membranes

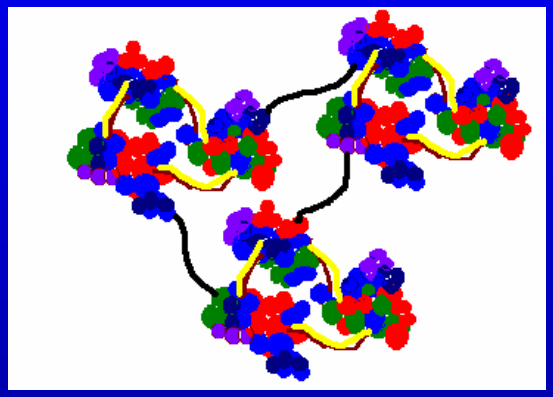
Entry angle favours attachment

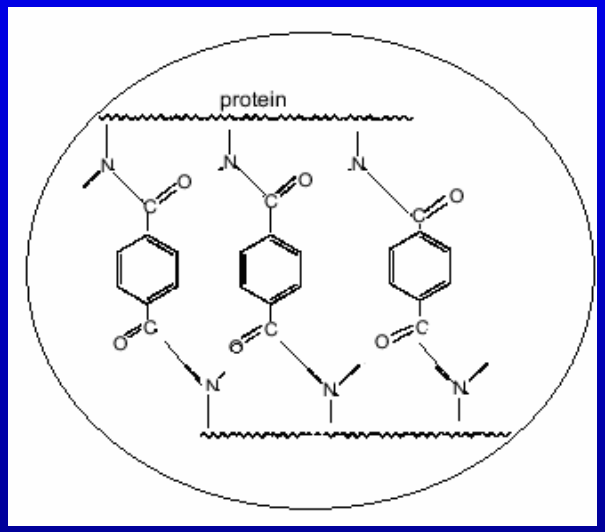
Interfacial protein crosslinking

20% (w/v) BSA (in buffer solution)

4% (w/v) Terephthaloyl chloride (in xylene)

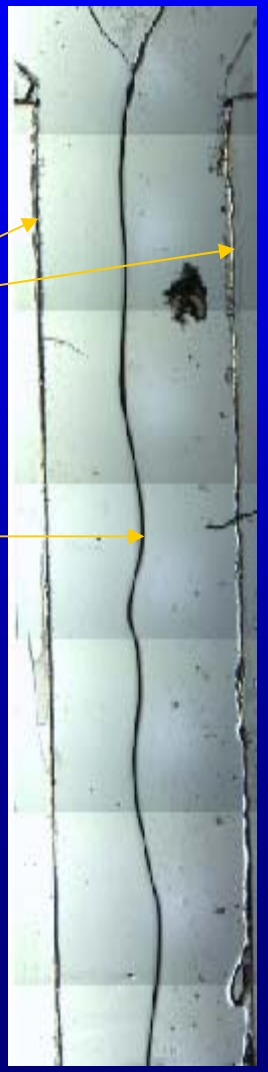


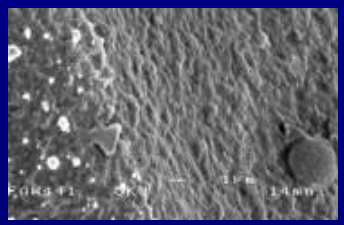




Channel walls

Crosslinked albumin membrane



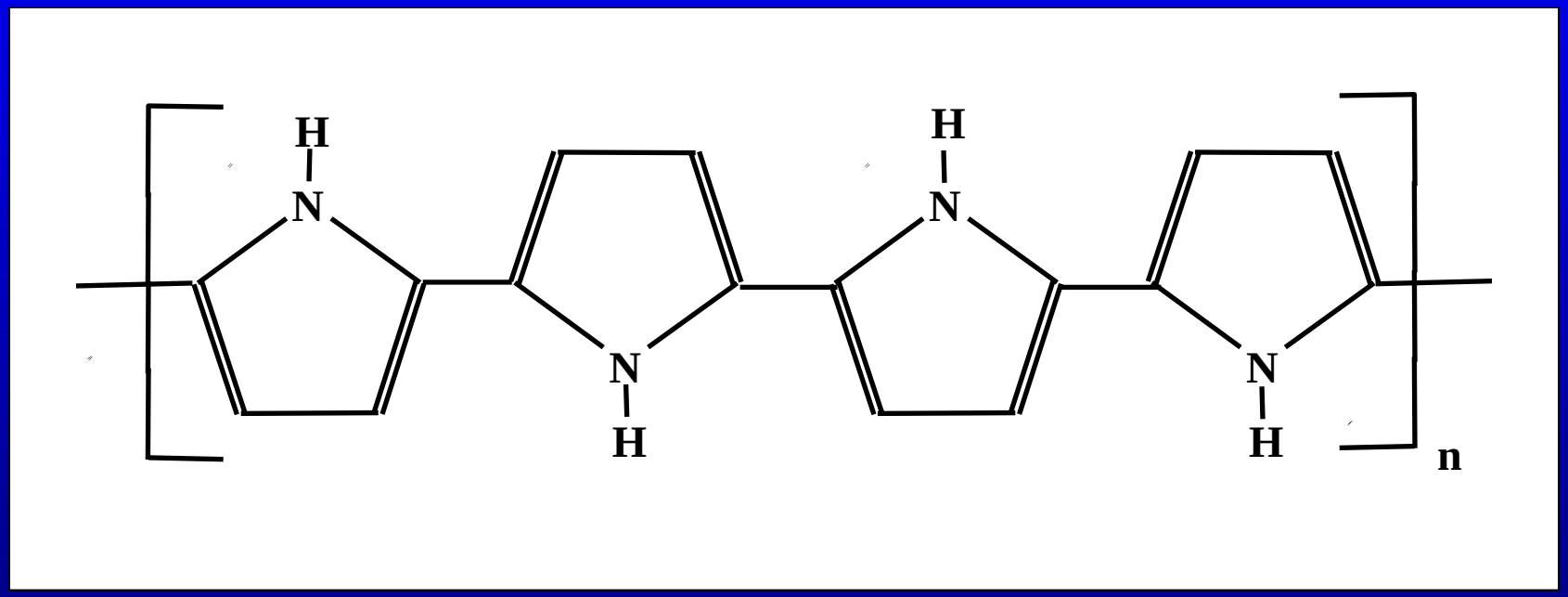


Flow rates:

1000 µl/min (xylene phase)

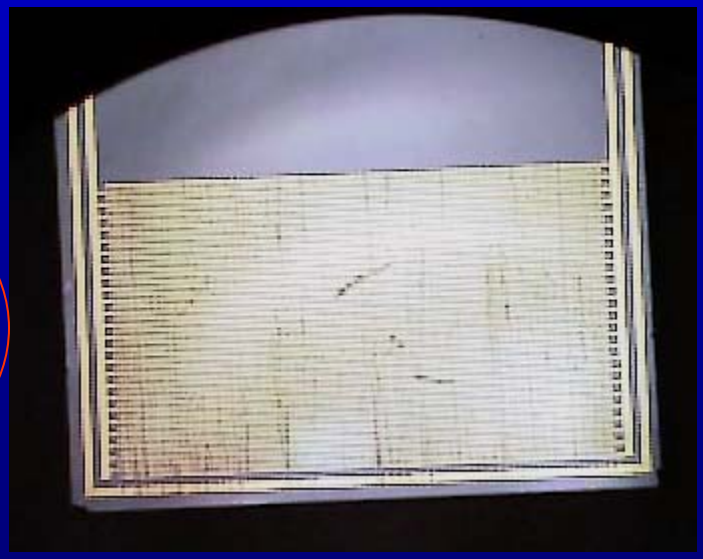
300 µl/min (aqueous phase)

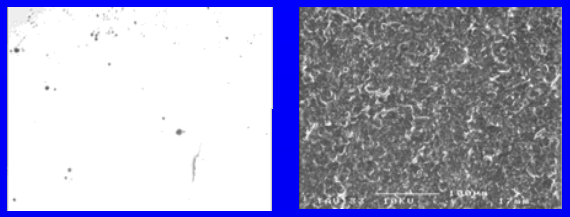
Poly(pyrrole)



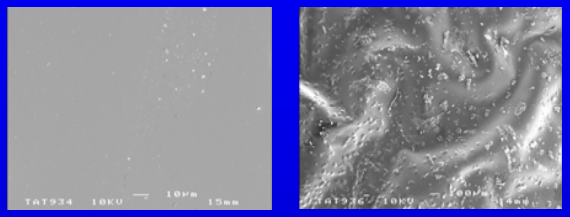
Two electrode impedance

- TWO ELECTRODE; Symmetrical, Accurate Instrumentation.
- Interdigitated Electrodes (IDE)
- Conducting Polymer as Reference- 20mV AC potential

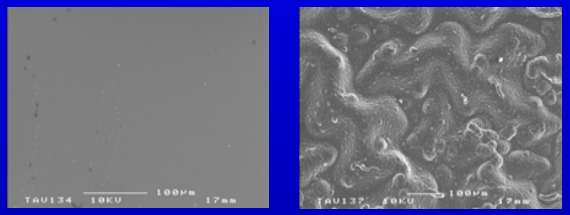




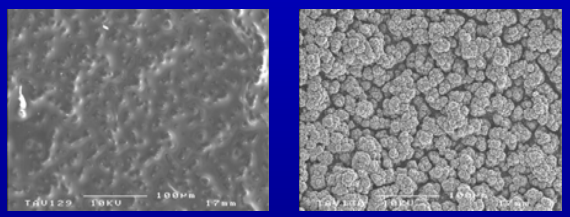
PPy/Cl



PPy/PVS



PPy/X



PPy/Col

Thin Films Left and Thick Films (Right)

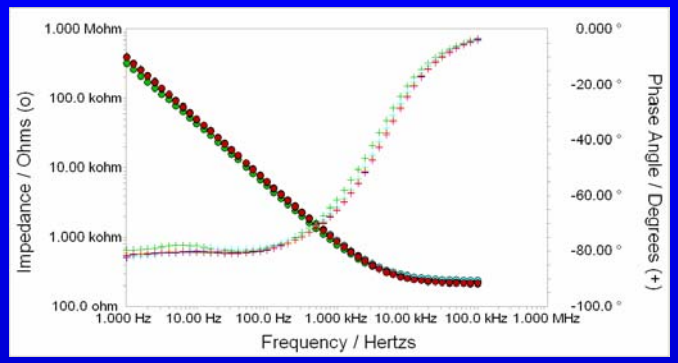
Cell growth on PPy films

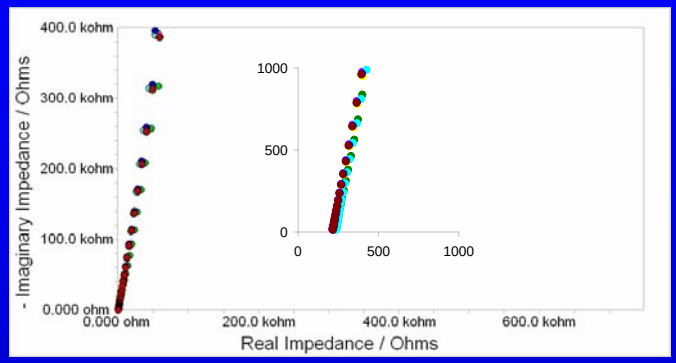
Examples of stained SVK14 keratinocytes on various substrates after 5 Days in culture (× 600)

From Growth Assays (AlamarBlue™ confirmed with Total Protein and ATP Quantitation) as well as Staining for Proliferation (PCNA), differentiation (K10) and Hyperproliferation (K16) Markers, Keratinocytes Growth was preferential on PPy substrates (PPy-Dermatan in particular) compared to bare gold

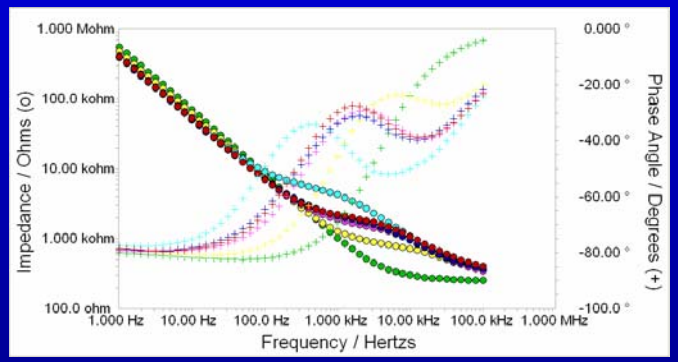
Sensing cells on gold

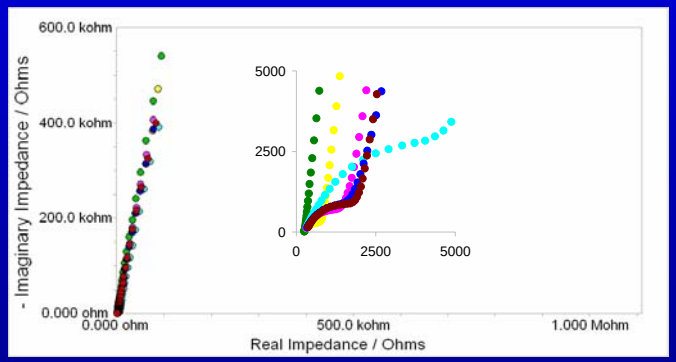
A





B



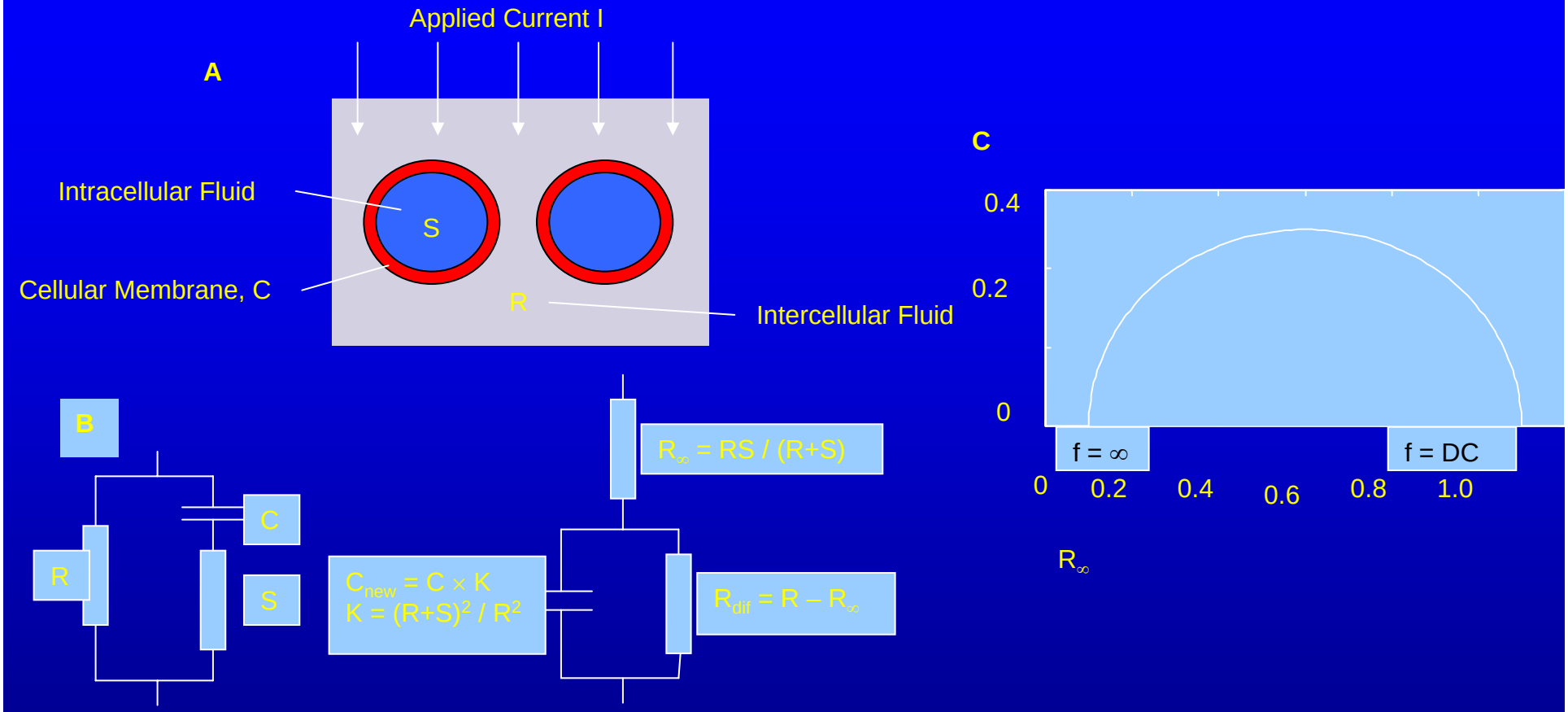


Day 0 Day 1 Day 2 Day 3 Day 4 Day 7

Representative Bode Plots (Left) and Complex Plane Plots (Right) of SVK14 Keratinocytes on Gold digit-Coated PC Coverslips

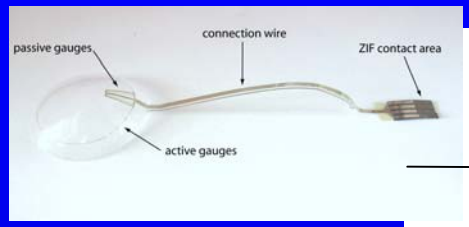
(A) Controls and (B) 4×10^5 Cells Seeded

Equivalent circuit analysis

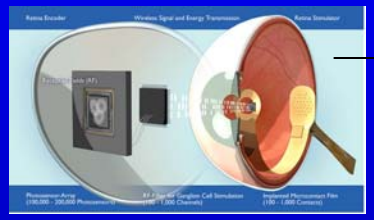


(A) Schematic of Cells in Tissue and Equivalent Electrical Components, (B) Equivalent Circuit for Tissue Model and Circuit of Equal Frequency Response Showing Relationship to (C) Cole Equation Parameters on Complex Plane Representation

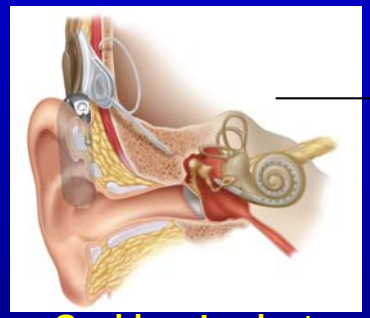
Adapted from Waterworth (2000), PhD Thesis, University of Sheffield



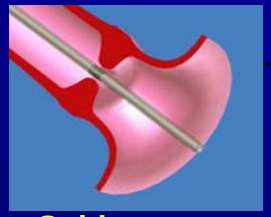
Glaucoma Sensor



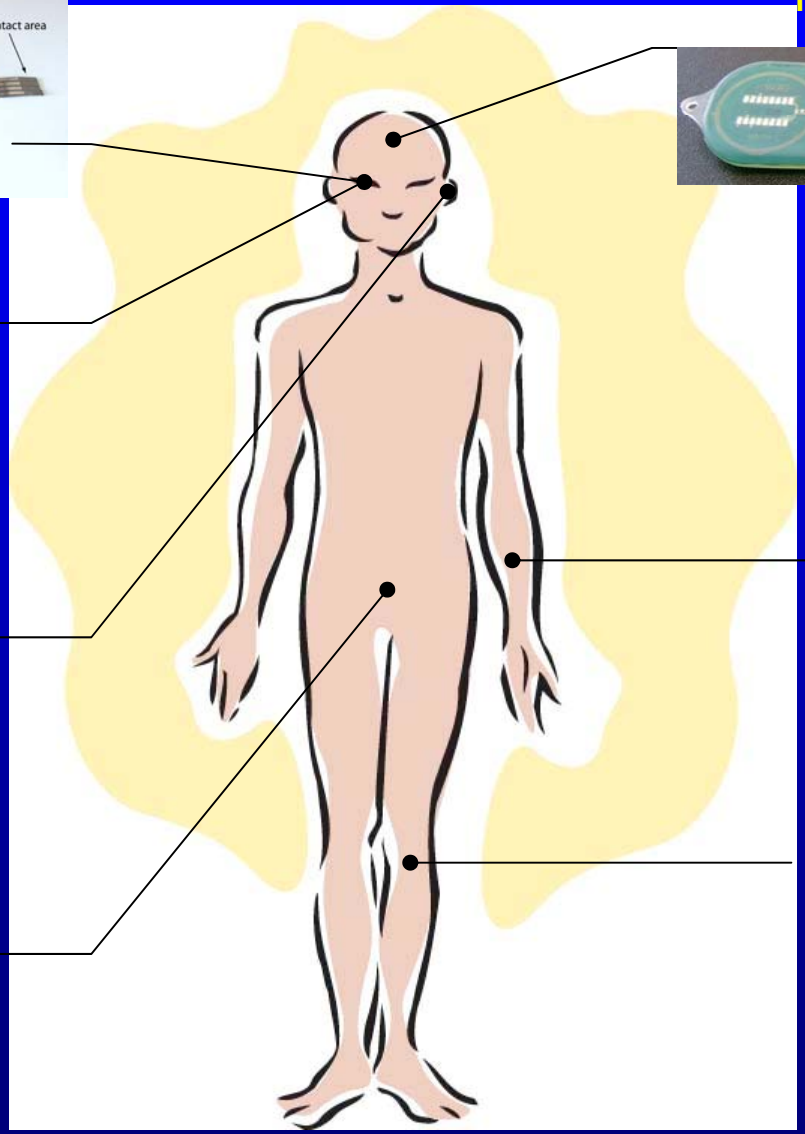
Retina Implant



Cochlear Implant

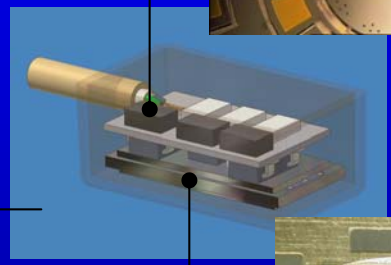


Sphincter sensor

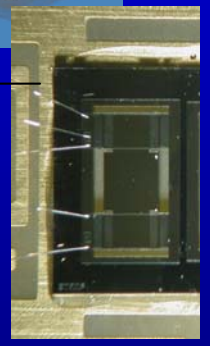


Intracranial Pressure Sensor

Gyro



IMU for Human Body Motion



Accelerometer

Functional Electrical Stimulation

What next?

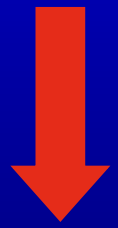
Multilayer membrane constructs

Reactive surfaces

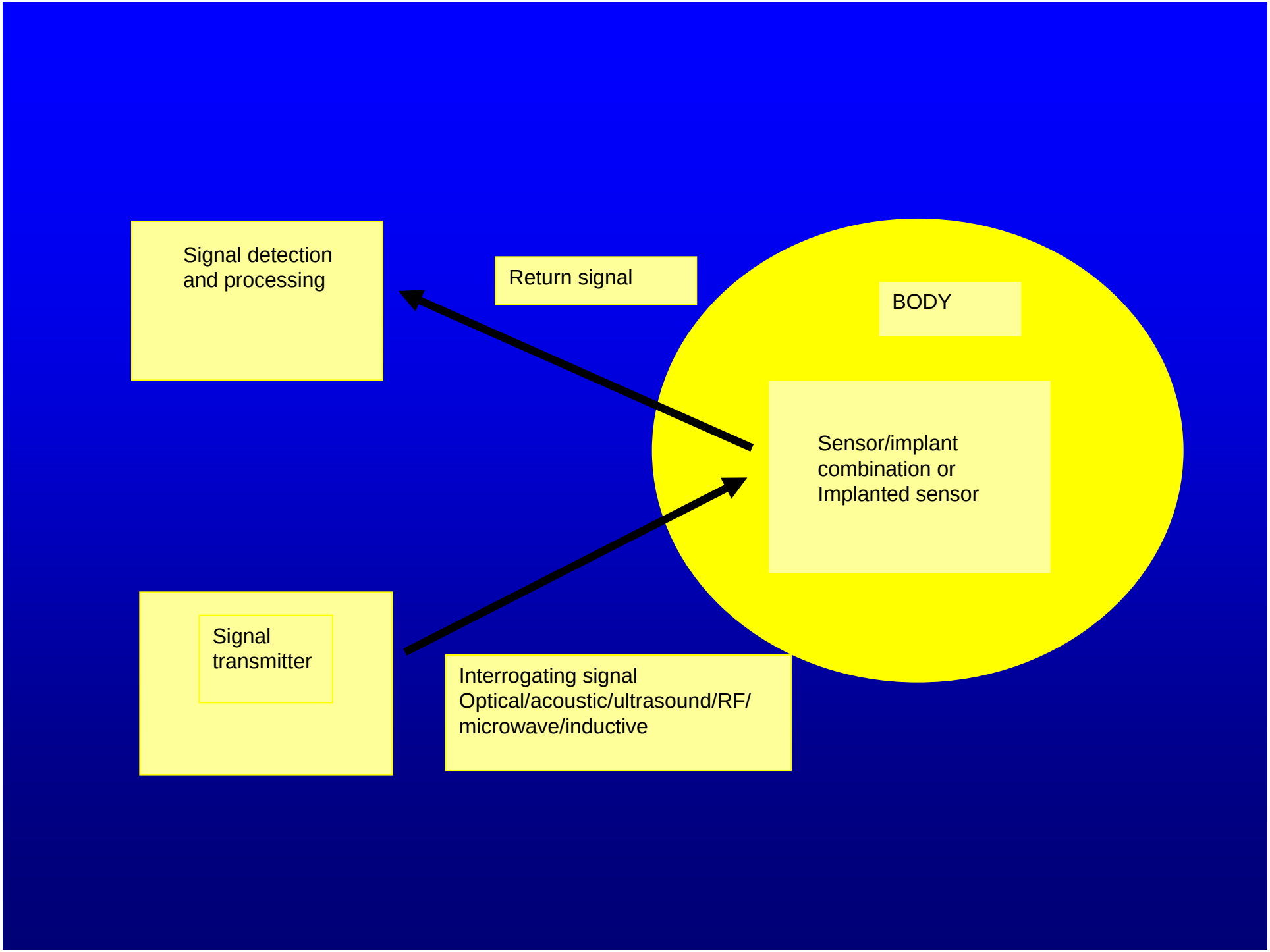
Charge / functional group control

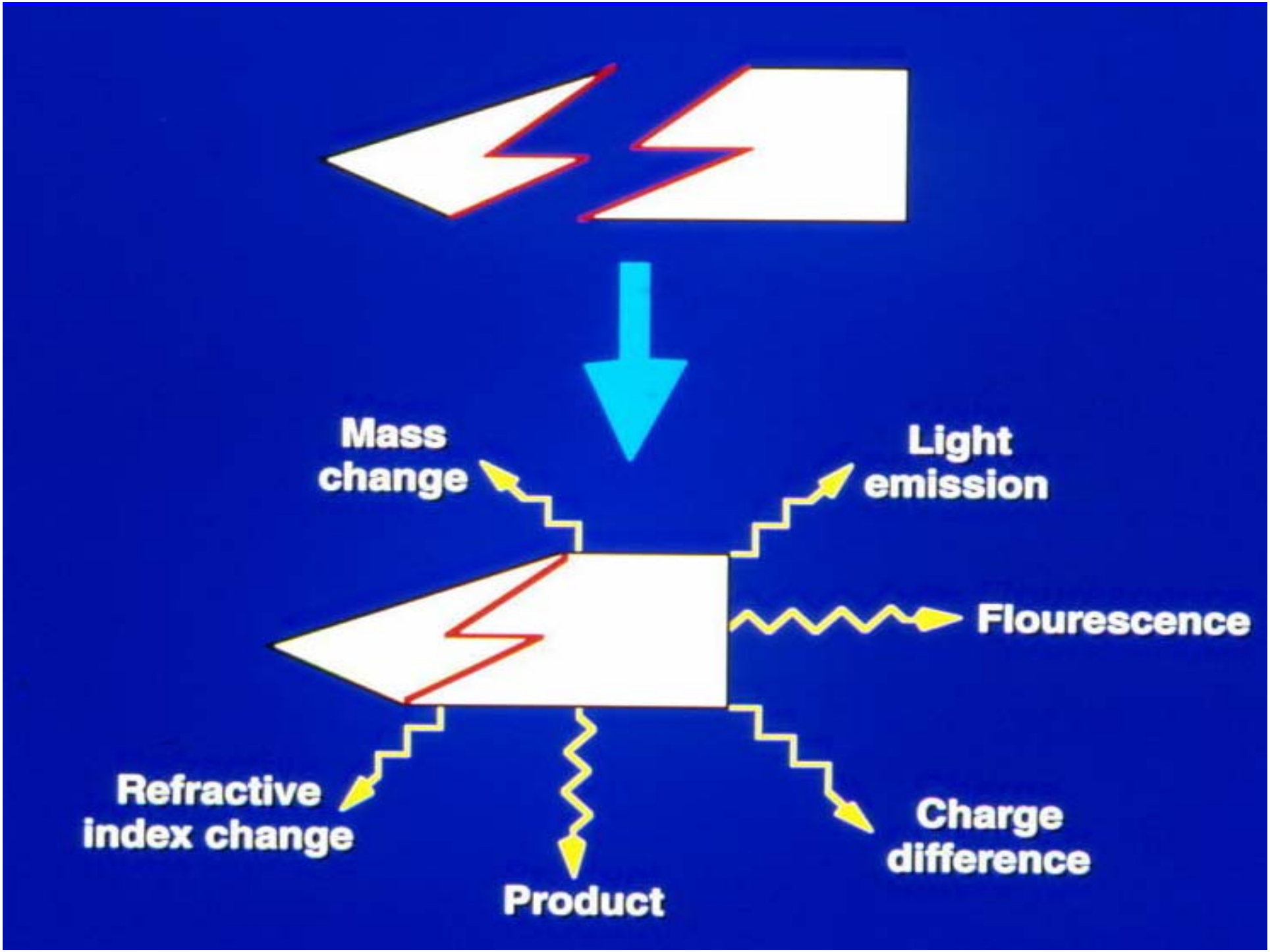
Membrane miniaturisation for MEMS

Biomimetic surfaces



Biomaterials

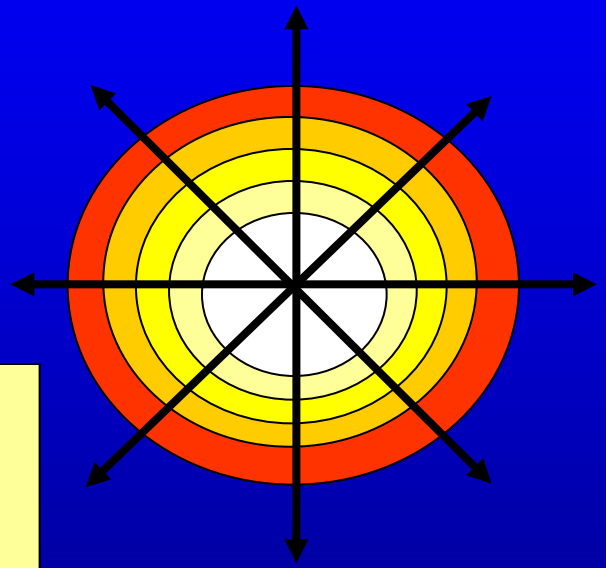

Signal detection and processing
Return signal
BODY
Sensor/implant combination or Implanted sensor
Signal transmitter
Interrogating signal Optical/acoustic/ultrasound/RF/ microwave/inductive


Mass change
Light emission
Flourescence
Refractive index change
Product
Charge difference

Retarding Factors
Medical conservation
Insufficient cost-benefit
Societal resistance to technology
Application complexity

Accelerating Factors
Improved technology diffusion
Reset national priorities
Basic science advances
Economy of scale up

Healthcare needs
Higher patient expectations
New surgical techniques
Reduced bed occupancy
Improved diagnostics
Over the counter diagnostics
Exploitation of New Biology

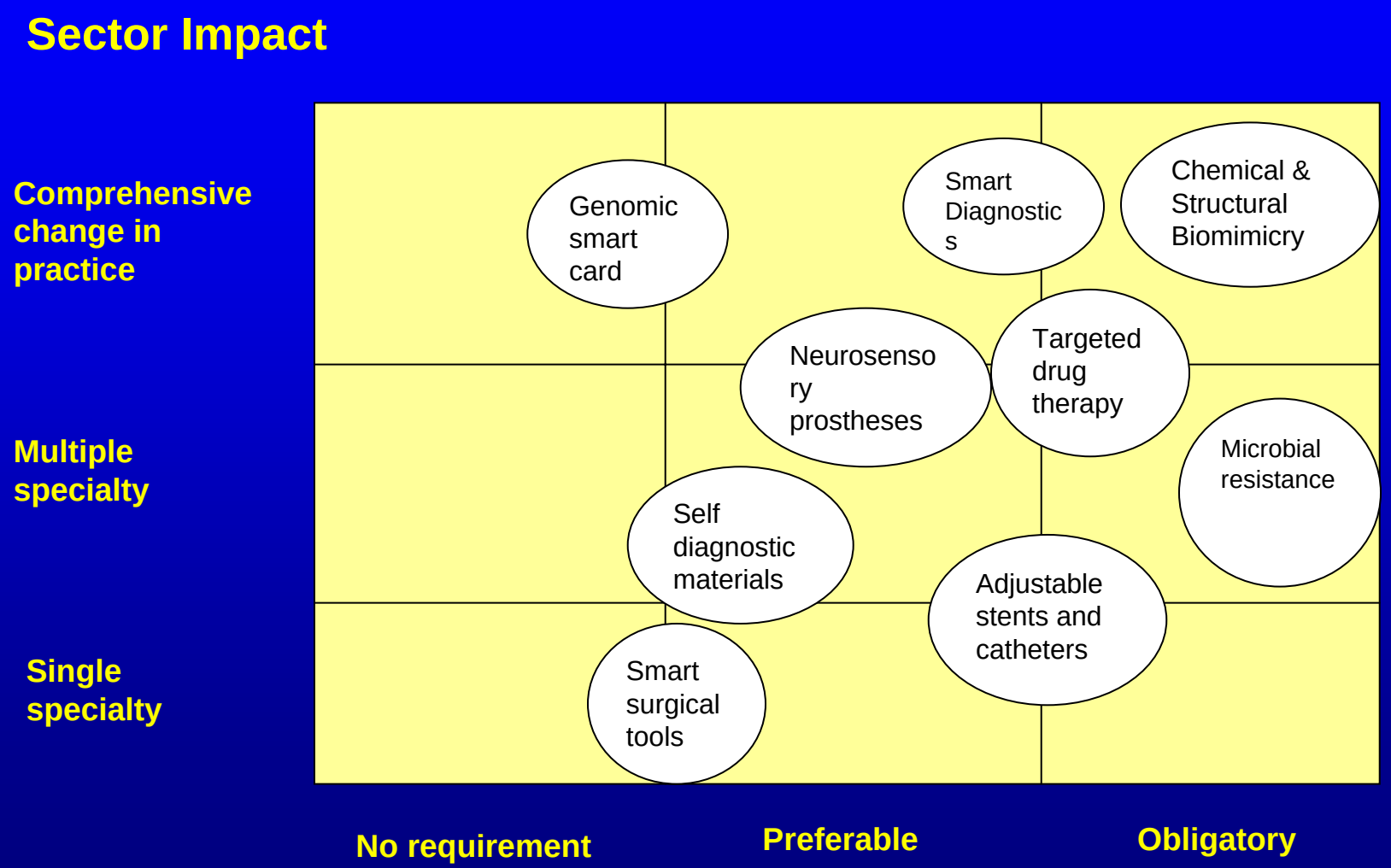


Regulatory Barriers
Safety thresholds lower
Multinational bodies
Sensational report
Ethical changes

Costs
Disproportionate increase.
Large work force requirement
Extended development time
Expensive QA

Competing technologies
New therapeutics
Existing biomaterials
MEMS devices
Microfabrication advances

New materials innovations



The UK Foresight Programme

http://www.iom3.org/foresight